A redescription of the external and buccopharyngeal morphology of the tadpole of *Ophryophryne microstoma* Boulenger, 1903 (Megophryidae)

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The external morphology and the buccopharyngeal features of the tadpole of *Ophryophryne microstoma* Boulenger, 1903 are redescribed. Morphometric data are provided. Few morphological deviations as compared to the previously described tadpoles from China are noted. The taxonomic status of the genus *Ophryophryne* within the Megophryidae is discussed in the light of the tadpole morphology.

INTRODUCTION

Ophryophryne Boulenger, 1903 is a small group of species from southeast Asia with a rather limited distribution, still little studied, and including now four species after a recent taxonomic revision of the genus (OHLER, 2003). The status of *Ophryophryne* has always been confused. Though its descriptor noticed its overall resemblance with members of the Pelobatidae (BOULENGER, 1903*a*), he included this genus within the Bufonidae (BOULENGER, 1903*b*) and it is still yet considered as such by some authors (NGUYEN & HO, 1996). It was then put within the Pelobatidae by NOBLE (1926). The status of the taxon *Ophryophryne* among the Megophryidae has already been discussed in previous works (NOBLE, 1926, DUBOIS, 1980, 1987; RAO & YANG, 1997; DUBOIS & OHLER, 1998). RAO & YANG (1997) rose *Ophryophryne*, *Atympanophrys* Tian & Hu, 1983, *Brachytarsophrys* Tian & Hu, 1983, *Megophrys* Kuhl & Van Hasselt, 1822 and *Xenophrys* Günther, 1864 to the generic level on the basis of cytological, morphological and ecological characters. DUBOIS (1980) put first *Ophryophryne* within the genus *Megophrys* then on the basis of new subdivisions within the genus *Megophrys* s. str. re-evaluated his proposition and gave *Ophryophryne* a generic status (DUBOIS, 1987). Though its position seems now to be clearly within the Megophryidae and the genus *Xenophrys* has

been identified as its sister-group by several authors (TIAN et al., 1985; RAO & YANG, 1997), its generic or subgeneric status (within the genus *Megophrys*) is still discussed (MATSUI *in* FROST, 1985; YE et al., 1993; MANTHEY & GROSSMANN; 1997)

Ophryophryne adults bear autapomorphies, which distinguish them from other members of *Megophrys* (sensu lato including four subgenera, i.e., *Atympanophrys*, *Brachytarsophrys*, *Megophrys*, *Xenophrys*), absence of maxillar and vomerine teeth and presence of a horizontal pupil. However, are these characters sufficient to consider *Ophryophryne* as a valid genus? Anuran tadpoles are, as a general rule, well intergenerically differentiated on the basis of the buccopharyngeal morphology (VIERTEL, 1982, Grosjean, unpublished data). The aim of this work is, besides a description of the morphology and buccopharyngeal features of the only known tadpole of the genus, to compare these data with those of known tadpoles of the four subgenera of the genus *Megophrys*, in order to discuss the status of *Ophryophryne* in the light of larval data.

MATERIALS AND METHODS

A total of 52 tadpoles, all *Ophryophryne microstoma* Boulenger, 1903, were collected in Ben En National Park, Thanh Hoa Province, Vietnam (19°30'-40'N, 105°21'-35'E) during August 1997. This national park is situated in a region of low hills surrounding the Song Muc Lake. Altitude ranges from 20 to 497 m above sea level, with most areas being below 200 m. The vegetation is that of a tropical semi-evergreen forest partly degraded by human activity. The climate is subtropical, with the heaviest rainfall between July and October. The average temperatures between 1961 and 1990, in the months of July and August, were 28.9 and 27.8°C respectively (TORDOFF et al., 1997).

Some tadpoles were preserved soon after capture whereas others were reared in bowls of 24 cm of average diameter and 10 cm depth, and were fed with baby fish food (TetraMin). Tadpoles in developmental stages 25-43 (GOSNER, 1960) were preserved in a mixture of equal parts of 4 % formaldehyde and 70 % ethanol (GRILLITSCH, 1984). Some tadpoles reached metamorphosis assuring the identity of the species and were preserved in the same solution. This material is deposited in the collections of the Muséum national d'Histoire naturelle of Paris (MNHN 1999.0521-0572).

Morphological terminology follows ALTIG & MC DIARMID (1999), developmental stages were determined according to GOSNER (1960) and terminology of buccopharyngeal features follows WASSERSUG (1976). Measurements were taken with a graduated ocular attached to a stereomicroscope except for the total length and the distance from opening of vent to tip of tail which were measured with a hand caliper. The distance from tip of snout to nares was not taken into account because the tip of snout was hidden beneath the oral funnel. For exact location of measurement landmarks, see GROSJEAN (2001: fig. 2), except for tip of snout which is taken from the point where the funnel originates with the head anteroventrally. Drawings were made with the aid of a camera lucida.

Preparation for SEM examination (JEOL JSM 840) comprised dehydratation (ethanol), critical-point-drying (liquid carbondioxide) and gold sputter surface coating.

RESULTS

ECOLOGICAL CONSIDERATIONS

The tadpoles studied were collected along a little stream running in the forest. The stream was 1-2 m wide and its depth ranged from a few centimetres in fast-flowing parts to up to 70 cm in the deepest pools. The bottom was mainly made up of rocks and stones, and of sand covered by dead leaves in the quiet pools. These tadpoles belong to the lotic-neustonic ecomorphological guild of ALTIG & JOHNSTON (1989). They were found in the lower part of the stream where the cover of vegetation was more open, in shallow water, hidden between the stones with their funnel open at the interface air-water. Tadpoles were also found near the banks of the stream, where the water ran between the stones, the tadpoles not being visible (as already noticed for tadpoles of the genus *Megophrys* by HORA, 1928) and in a little natural dam made up by branches and dead leaves. All developmental stages (from stage 25 to imago) were found all together at the same time and in the same part of the stream. This suggests that the mating and/or spawning period might be prolonged (parsimonious assumption) or that larval development is very slow and extends all over the year rather than being limited to a circumscribed period (in accordance with other observations). Indeed the tadpoles reared for one month did not show evolution (except for the latest stages). Slow development is not rare among Megophryidae and was observed in other genera like *Leptobrachium* Tschudi, 1838, *Leptolalax* Dubois, 1980, *Scutiger* Theobald, 1868 and *Oreolalax* Myers & Leviton, 1962 (SMITH, 1917; LIU & HU, 1960, CHEN et al., 1984, ZHAO et al., 1994; pers. obs.) and in rheophilous species in general. Few tadpoles of *Leptolalax* sp. were found among them. Numerous tadpoles of *Leptobrachium chapaense* (Bourret, 1937) lived in the pools of the same stream but not in the same niche (no tadpole of *Ophryophryne microstoma* was found in pools). Clutches of *Rana (Sylvirana) nigrovittata* (Blyth, 1855) were found in crab holes, in the bank.

During the two months spent in the field, the reproduction of the species which produced the tadpoles was not observed: advertisement calls were not heard and neither mates in amplexus nor egg clutches were found. Non-calling adults of *O. microstoma* were occasionally found among the vegetation of the bank and on emerged rocks in the stream bed.

DESCRIPTION OF TADPOLE

External morphology

Gross morphometric parameters (SVL and TL) of all tadpoles are presented in table 1. The following description is based on four tadpoles at stages 35-38 (MNHN 1999.0532-0535), except where specified. Detailed morphometric data are presented in table 2. In dorsal view (fig. 1a), body elliptical. Eyes of moderate size (diameter about 0.1 time body length), bulging, separated by a distance which equals about 1.3 times the internarial distance, directed and positioned laterally, visible in ventral view. Nares tubular, of moderate size, directed laterally

Table 1 Variation of snout-vent length (SVL) and total length (TL) with stage (GOSNER, 1960) in tadpoles of *Ophryophryne microstoma*. Number of tadpoles (*n*) examined, mean value ± standard deviation in mm (range in parentheses).

Stage	*n*	SVL	TL
25	24	5.87 ± 0.74 (5.06-8 55)	17.49 ± 2.19 (15.00-24.30)
26	7	7.83 ± 0.50 (7.06-8 55)	22.75 ± 1.34 (20.65-24.75)
27	2	8.62 ± 0.28 (8.42-8.82)	26.35 ± 0.07 (26.30-26.40)
28	2	9.14 ± 1.21 (8.29-10.00)	27.45 ± 2.05 (26.00-28.90)
31	3	9.56 ± 0.20 (9.34-9.74)	27.28 ± 0.88 (26.35-28.10)
34	1	10 92	31.05
35	1	9 47	28.10
37	2	10.66 ± 0.00 (10 66-10.66)	30.70 ± 3.54 (28.20-33.20)
38	1	10 66	32.40
40	1	11 18	31.60
41	5	9.87 ± 0.57 (9.21-10.53)	28.42 ± 1.54 (25.85-29.65)
42	1	10 26	29.75
43	1	11 18	25.55

Table 2 – Morphometric data for tadpoles of *Ophryophryne microstoma* in avanced developmental stages (35-38, GOSNER, 1960) BH, maximum body height, BW, maximum body width, ED, maximum eye diameter; HT, maximum tail height, LF, maximum height of lower tail fin, MNHN, collection number, Muséum national d'Histoire naturelle, Paris, *nm*, no measurement; NN, internarial distance, NP, naro-pupillar distance; PP, interpupillar distance; SS, distance from tip of snout to opening of spiracle; SU, distance from tip of snout to insertion of upper tail fin; SVL, snout-vent length; TL, total length, UF maximum height of upper tail fin; VT, distance from vent opening to tip of tail.

Stage	MNHN	SVL	TL	SS	SU	VT	HT	UF
35	1999.0535	9.47	28.10	4.21	11.71	19.30	4 99	1 28
37	1999.0534	10 66	28.20	4.67	10.79	18 60	4 86	1 24
37	1999.0533	10 66	33.20	4.44	14.34	21.50	4.86	1.31
38	1999.0532	10 66	32.40	4.93	8 03	21.70	4 99	1 34
Stage	MNHN	LF	BH	BW	ED	PP	NN	NP
35	1999.0535	1.40	*nm*	*nm*	1 01	3.24	2 61	0 75
37	1999.0534	1.18	4 15	4.73	1 09	3.50	2.72	0 93
37	1999 0533	1.18	4.15	4.73	1 09	3.64	2.71	0 93
38	1999 0532	1.24	4.08	4.99	1 10	3 48	2 49	0 96

and horizontally, and positioned rather dorsally, closer to pupils than to tip of snout. In profile (fig. 1b), body depressed. Spiracle sinistral, conical, very short, attached to the body wall except its tip which is free, positioned just beneath the longitudinal axis, oriented posteriorly. Spiracular opening situated slightly closer to pupil than to the end of the body, and at a level situated just beneath the apex of the caudal myotomes (fig. 1b) to between the apex of caudal myotomes and the hindlimbs, its opening from rounded to oval. Tail musculature strong, gradually tapering, almost reaching tail tip (very near to the end). Tail fins shallow, moderately developed, not extending onto body, dorsal fin slightly higher than ventral fin in the distal half of the tail; free margin of dorsal fin horizontal and very shallow in the proximal half of the tail then following tail musculature; free margin of ventral fin parallel to tail musculature. Point of maximum height of tail located just before halfway of the tail, in the proximal part, tail tip bluntly pointed. Vent tube of moderate size, medial, tubular (often slightly bulging in the middle), directed posteriorly, not linked to ventral tail fin, opening medial. Neither skin glands nor neuromasts visible.

Oral disk subterminal (fig. 2), lips expanded vertically forming a dorsally oriented funnel; lateral corners pronounced; upper lip smaller than lower; lips lacking keratodonts, but furnished with a few short, low ridges (variable number among specimens), more densely arranged on the upper labium than on the lower one: arrayed in 20 longitudinal rows (in mean) and 2 (on the upper labium) to 4 (on the lower labium) transversal rows (without clear limits), no marginal papillae. Width of expanded funnel about 50 % of body length (35 % when folded, which is often the case in the preserved specimens). At stage 40 the funnel began to be resorbed, at stage 42 the posterior part of the funnel disappeared while the anterior part still persisted. At stage 43 the funnel entirely disappeared. Lower jaw sheath (fig. 3a) straight anteriorly, radically curved backward laterally, entirely white, its free margin bearing fine, pointed, hair-like serrations; upper jaw sheath (fig. 3b) nearly straight, notched medially, bearing fine and elongate serrations only in the medial third of its free margin, brown grey with a white margin; both beaks soft.

Colour in preservative

Dorsal side of body and upper part of flanks brownish-khaki. Lower part of flanks weakly mottled with the same colour; ventral side of head (from snout to posterior part of eyes) intensely coloured. Belly white, intestine not visible through body wall. Caudal muscle weakly coloured (more on upper than on lower portion, with emphasis on the myotome apexes), tail fins translucent with few spots (more on upper than on lower fin); half to a quarter of distal part of tail often intensively coloured in dark brown. Oral funnel greyish with brownish-khaki papillae. There is considerable intrapopulational variation in colour: certain individuals are almost unpigmented whereas others are strongly coloured, others have a tail strongly coloured except in the distal quarter.

Tadpole at stage 45 (just metamorphosed, MNHN 1999.0524, SVL 11.97 mm) in preservative. Head and dorsum brown, flanks and dorsal part of thigh and tibia with large white tubercles. Dorsal folds present. Supratympanic fold underlined with white above and black below, the black line extending as far as the armpit. Limbs white below, faintly tinted with brown above. The above features began to appear from stage 42 on.

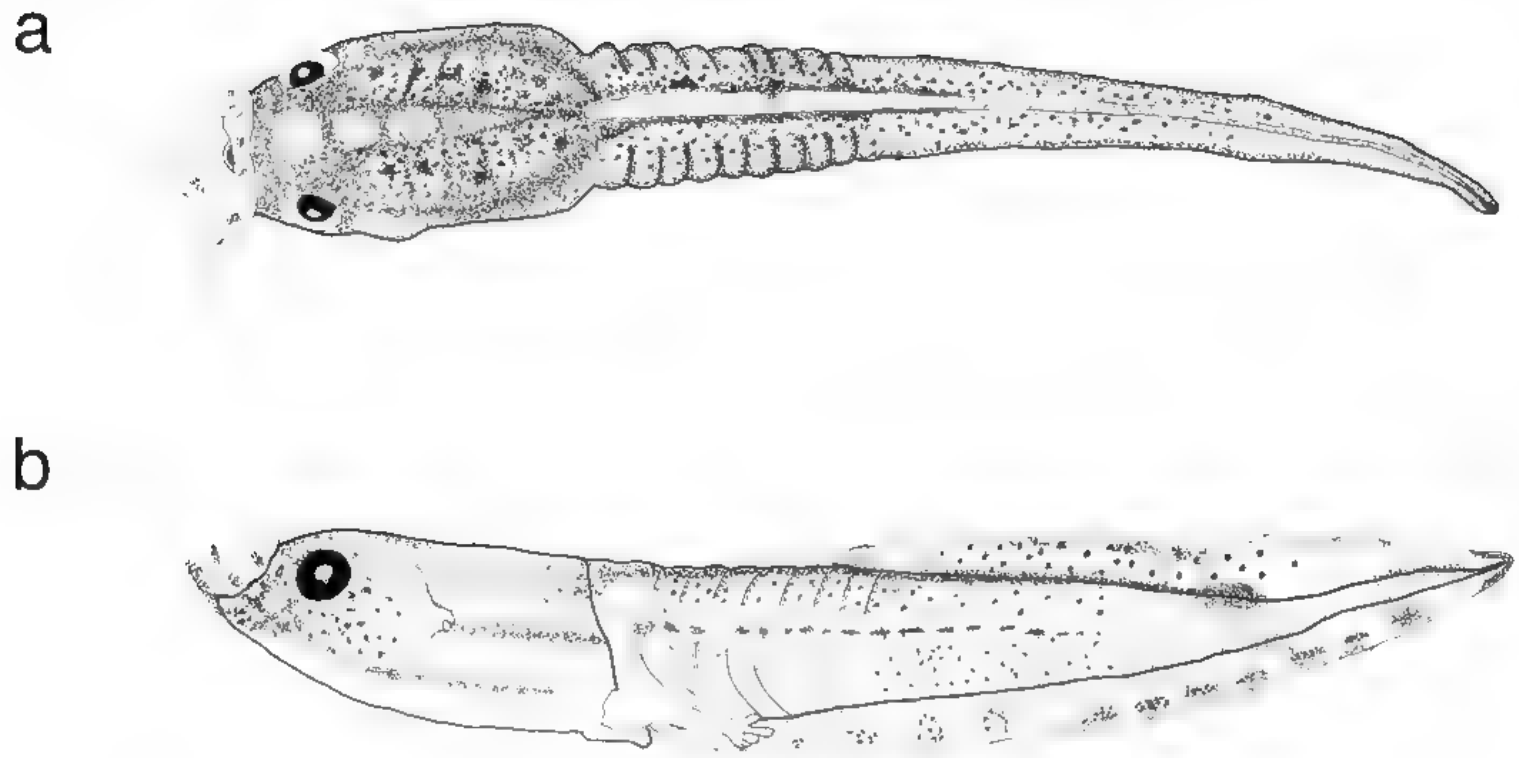

Fig 1 *Ophryophryne microstoma* (stage 38, TL 32,1 mm, MNHN 1999.0532) a, dorsal view; b, lateral view.

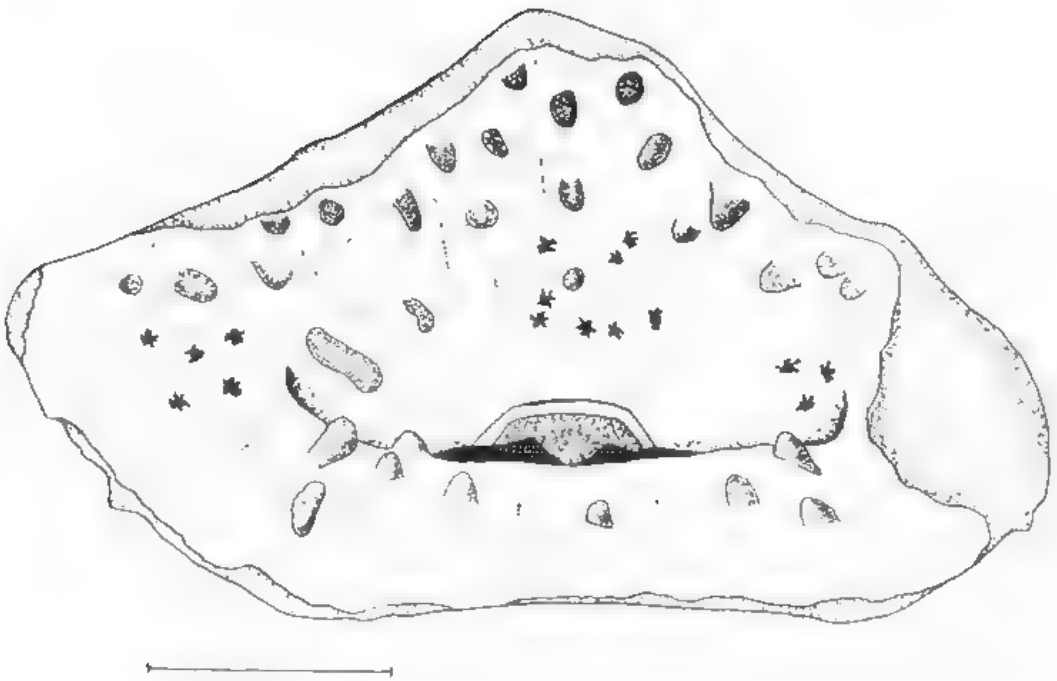

Fig. 2 Funnel like mouth of *Ophryophryne microstoma* (stage 38, MNHN 1999 0532) Scale line 1 mm

Internal buccal features

The description is based on a single tadpole at stage 37 (MNHN 1999.0534).

Buccal floor (fig. 4a), a soft lower jaw sheath present at entry of buccal cavity, straight anteriorly, radically curved backward laterally, a nodule on the inner wall of the beak at the level of curvatures. Prelingual arena deep, with two successive pairs of palps directed medially, followed by a fifth palp in median position These structures are thick, fleshy and smooth lobes, concave anteriorly, the first pair reaching almost the lower jaw sheath being visible without dissection. Tongue anlage distinct, not very prominent, drop-shaped, positioned on an elevated mass, lingual papillae absent Buccal floor arena forming an elongate oval, in a depression, without buccal floor arena papillae but its anterior half bounded laterally by a thick ridge on each side, these two ridges merging with the elevated part bearing the tongue anlage anteriorly The ridges ending posteriorly in front of the buccal pockets with elevated knobs each wrapped by an anteriorly concave flap, posterior half of arena bounded by an undulating ridge on each side. Anterior part of arena with a median groove of about a third of the length of the buccal floor arena, 2-5 pustules on each side between the ridges and the median groove, posterior part of arena lacking ornamentations. Buccal pockets short, transversely oriented, arched anteriorly, with fine openings, area anterior to buccal pockets with 1-3 pustules on each side, area posterior to buccal pockets with less than 10 small

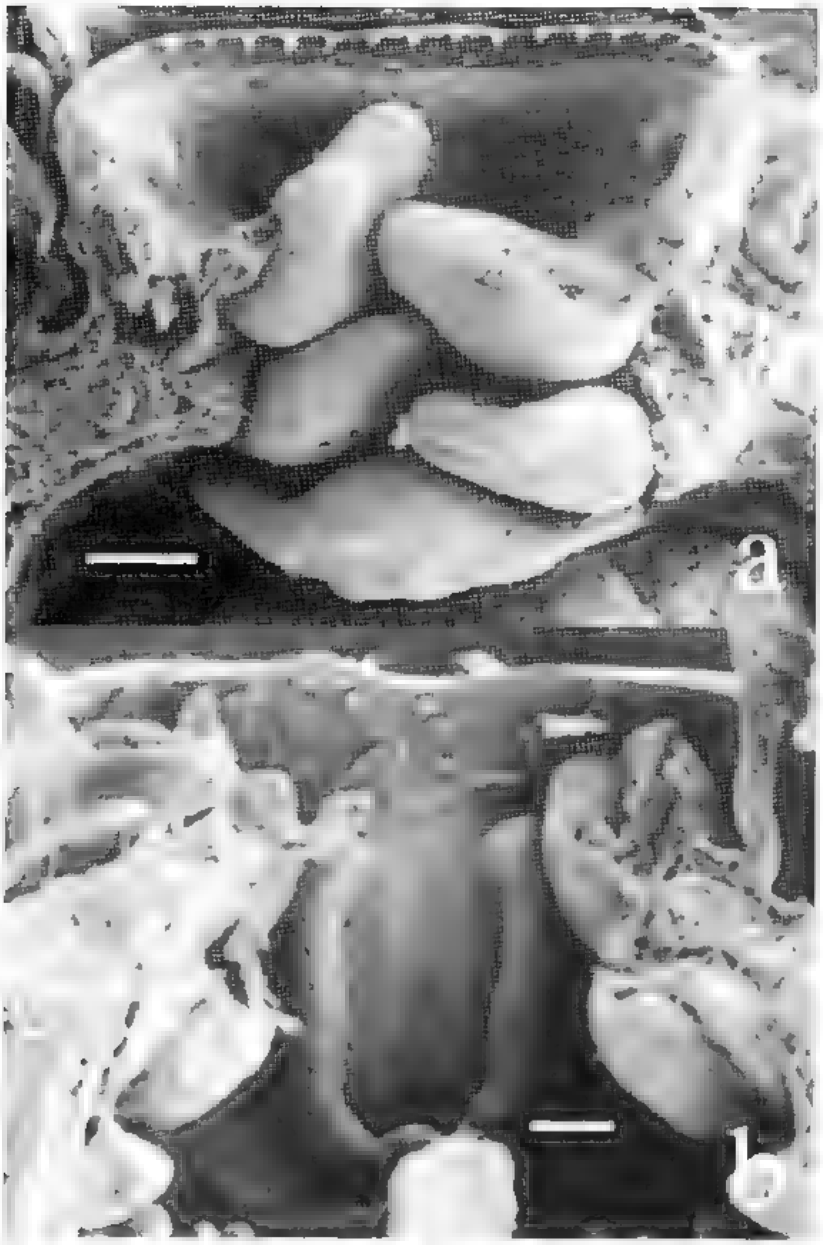

Fig. 3 Close-up view of the jaw sheaths of *Ophryophryne microstoma* (stage 37, MNHN 1999.0534) a, lower part; b, upper part. Scale line: 0.1 mm

papillae. Ventral velum slightly undulated, its medial part extending backward; two minor projections laterally, medial notch absent; spicular supports present laterally, secretory pits of ventral velum absent, glottis slightly exposed. Branchial baskets large; a single common filter cavity on each side, filter rows wide with tertiary or higher-order ramifications, mesh size about 100 μm.

Fig. 4. *Ophryophryne microstoma* (stage 37, MNHN 1999.0534). a, buccal floor arena; b, buccal roof arena.

Buccal roof (fig. 4b) upper jaw sheath nearly straight, bearing fine and elongate serrations only in the medial third. Prenarial arena wide, trapezoidal, in medial position a large U-shaped ridge with a posteromedial knob, its anterior arms almost reaching the beak; a little knob just posterior to the U-shaped ridge (hidden by the median ridge), on the wall of the prenarial arena two pairs of well pronounced dorsoventrally compressed ridges aligned longitudinally. Choanae transverse, short; anterior narial wall smooth, with a short, stocky papilla on the internal end and another even stockier on the external end, narial valve smooth with a small stocky triangular projection located rather medially. Postnarial arena bounded laterally by a pair of pronounced ridges (which could be the homologue of postnarial papillae), fused anteriorly with the medial posterior wall of choanae, ending behind the medial ridge, their posterior ends curved medially. Median ridge a tall straight projection directed anteriorly plus three little prominent lobes, reaching as far as the posteromedial knob of the prenarial U-shaped ridge. Postnarial arena filled by the body of the median ridge. Lateral ridge papillae a large dorsoventrally compressed flap; above their anterior part a similar but smaller flap. Buccal roof arena with about fifty pustules arranged more densely posteriorly, no buccal roof arena papillae but two large nodules fused on each side bounding anterolaterally the buccal roof arena and abutting the ridges lateral to the medial ridge. About ten pustules on each lateral wall of the buccal roof, at the level of the anterior half of the buccal roof arena. Posterolateral ridges present but not very prominent laterally and medially, and rather distinct lateromedially. Glandular zone rather indistinct, absent medially; secretory pits absent. Dorsal velum curved ventrally, interrupted medially.

Discussion

Among the four species in the genus *Ophryophryne* known at present, only the tadpole of *O. microstoma* has been described previously. I redescribe here the tadpole of the species based on specimens belonging to a much more southern population.

Liu & Hu (1962) reported on the external morphology of the tadpole from Kwangsi Province, China. These authors did not specify the developmental stage of the tadpole upon which their description was based but just noticed that it bore buds of hind limbs and had a total length of 33 mm. The only tadpole in our sample which reached this size was a tadpole of stage 37. Hence, tadpoles in equivalent developmental stages were larger in the Chinese sample than in the Vietnamese sample. The other characters differing between the two samples were: oral disk bigger, eyes larger and tip of tail rounded (not bluntly pointed as in this sample) in the Chinese population. Differences were also observed in the number of papillae of the funnel: 15 longitudinal rows and three transversal rows in the Chinese population, 20 longitudinal rows (in mean) and 2-4 transversal rows (without clear limits) in our sample. Finally, the coloration varied in a few points: in the tadpole of the Vietnamese population the upper fin was rimmed with dark margins in its anterior part and the anterior part of the lower fin was coloured with large dark marks whereas it was white in the Chinese one. The imago collected by Liu & Hu (1962) was comparatively large (14 mm body length and 3.5 mm tail, whereas an imago entirely metamorphosed collected in the Vietnamese population was about 12 mm in length). However in both cases the adult characters appeared

early since the skin folds and the tubercles were present before resorption of tail. The eyelid processes seemed to appear after total resorption of tail.

The buccopharyngeal features of a specimen from Longjin, Guangxi Province, China were reported by HUANG et al. (1991). Their illustration showed some differences with our data. In the buccal floor of the HUANG et al. (1991) specimen the posterior pair of palps as well as the single medial palp are shorter than in our specimen and a medial notch is present. Within the buccal roof, our specimen had a bigger prenarial ridge, two papillae on anterior narial wall and another on the narial valve whereas only one was present on the anterior narial wall in the Longjin specimen; moreover, pustulations in the buccal roof arena were present in the Vietnamese tadpole.

The tadpoles of the genus *Megophrys* sensu lato (including the subgenera *Atympanophrys*, *Brachytarsophrys*, *Megophrys* and *Xenophrys*) have been little studied and their detailed descriptions are rare: (1) for the external morphology, *M. (X.) aceras* (Boulenger, 1903) (LAIDLAW, 1900, ANNANDALE, 1912, 1917, SMITH, 1926 [all under the name *M. montana*]), *M. (X.) major* (Boulenger, 1908) (ANNANDALE, 1912), *M. (X.) parva* (Boulenger, 1893) (ANNANDALE, 1912), *M. (X.) boettgeri* (Boulenger, 1899) (ANNANDALE, 1917; POPE, 1931; LIU, 1940), *M. (X.) longipes* (Boulenger, 1885) (LEONG & CHOU, 1998), *M. (X.) minor* Stejneger, 1926 (LIU, 1950), *M. (M.) montana* Kuhl & Van Hasselt, 1822 (BOULENGER, 1908), *M. (M.) nasuta* (Schlegel, 1858) (INGER, 1985); (2) for the buccopharyngeal features, *M. (B.) carinensis* (Boulenger, 1899) (HUANG et al., 1991), *M. (X.) minor* (WASSERSUG, 1980; HUANG et al., 1991), *M. (M.) nasuta* (INGER, 1985), *M. (X.) omeimontis* Liu, 1950 (HUANG et al., 1991) and *M. (A.) shapingensis* Liu, 1950 (HUANG et al., 1991). The tadpoles of the genus *Megophrys* sensu lato are very similar to those of *Ophryophryne* and nothing allows to distinguish them externally. The most peculiar structure of these animals, the funnel-like oral disc surrounding the mouth, is very conservative and its typical morphology is encountered in all species. The small differences reported in terms of variation in number of rows or of ridges are not greater between *Ophryophryne* and *Megophrys* sensu lato than between the members of the genus *Megophrys* sensu lato themselves (Grosjean, unpublished data). Of all the species on which the width of the funnel was measured, the tadpole of *Ophryophryne microstoma* is the one which possesses the smallest [50 % of SVL vs. 74.4 % in *M. minor* (LIU, 1950) and *M. boettgeri* (LIU, 1940), and 82.3 % in *M. aceras* (LAIDLAW, 1900)]. LEONG & CHOU (1998) did not give a measurement comparable but noticed that the funnel of *M. longipes* is the largest of all the species known. The tadpoles of these two genera bear another peculiar structure, unique among the Megophryidae: soft jaw sheaths with long serrations at the opening of the mouth (INGER, 1985, HUANG et al., 1991, this paper). This structure, although externally visible, is not always conspicuous. It is now largely accepted that buccopharyngeal features are very conservative within genus (e.g. VIERTEL, 1982) as, e.g., within the Megophryidae (INGER, 1983, for a taxonomic review of three genera with larval characters, HUANG et al., 1991). Each megophryid genus has a typical buccopharyngeal morphology, which distinguishes it clearly from all other megophryid genera. However, in contradiction to HUANG et al. (1991)'s conclusion (not detailed in their work), I failed to find any significant differences in their buccopharyngeal features. The morphology of these tadpoles does not contribute to clarify the status of *Ophryophryne*, but the lack of clear differences between *Ophryophryne* and the species of the four taxa included in *Megophrys* (sensu lato) does not justify a different taxonomical level for the former. A recent study based on cytology, morphology and ecology

(RAO & YANG, 1997) suggested two clades within this group, one including large species (*Atympanophrys*, *Brachytarsophrys* and *Megophrys*), the other including small and moderate-sized species (*Ophryophryne* and *Xenophrys*). Furthermore, RAO & YANG (1997) proposed that these five taxa should be risen to generic level. The results of the present study do not conflict with the opinion of these authors.

RÉSUMÉ

La morphologie externe, incluant des données morphométriques et l'anatomie buccopharyngée du têtard d'*Ophryophryne microstoma* Boulenger, 1903 sont présentées. Les individus de cet échantillon présentent quelques différences morphologiques avec des têtards rapportés à cette espèce mais appartenant à d'autres populations. Le statut taxinomique du genre *Ophryophryne* au sein des Megophryidae est discuté à la lumière des caractéristiques morphologiques et buccopharyngées du têtard d'*Ophryophryne microstoma* et de celles du genre *Megophrys* au sens large publiés dans la littérature.

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